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Aggregating the Message to Control the Cell Cycle

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Localization of messenger RNAs (mRNAs) enables the precise regulation of protein expression in space and time. In this issue of *Developmental Cell*, Lee et al. (2013) report that the RNA-binding protein Whi3 spatially constrains a cyclin-encoding mRNA in the cytoplasm of multinucleate cells, thus allowing independent cell-cycle control of individual nuclei.

Asymmetric messenger RNA (mRNA) localization is much more common than previously anticipated. In the developing fly embryo, more than 70% of mRNAs are unevenly distributed (Lécuyer et al., 2007). Much insight about the mechanisms of mRNA localization has been gained from developing embryos, where mRNAs are targeted to specific locations to organize tissues and organs and instruct body plan formation. However, heterogeneous mRNA localization is not limited to embryogenesis. It has also been observed in the growing protrusions of migrating fibroblasts or the extended processes of neurons. Thus, mRNA localization appears to be a very potent way to spatially and temporally control the expression of genes. In this issue of *Developmental Cell*, Lee et al. (2013) expand the universe of mRNA localization by demonstrating in the yeast *Ashbya gossypii* that a specific cyclin-encoding mRNA is spatially constrained by the RNA-binding protein Whi3, thus allowing asynchronous cell-cycle timing in multinucleate cells. They further propose that mRNA clustering is dependent on an aggregation-prone polyglutamine sequence in Whi3.

Localization of mRNAs is a multistep process that involves export from the nucleus, active transport by molecular motors, and localized anchorage. Once an mRNA arrives at its destination, it can respond to specific cues, allowing the localized production of proteins and the spatially confined remodeling of cellular microdomains. How do cells achieve this remarkable task? Asymmetrically distributed mRNAs contain *cis*-acting localization sequences (also known as zip codes) that are decoded by a functionally and structurally diverse group of RNA-binding proteins. As a consequence—and in contrast to many textbook representations—mRNAs are wrapped into a dense coat of RNA-binding proteins. Once formed, such ribonucleoprotein (RNP) particles can interact with other particles to grow into dynamic granules, which sometimes reach sizes of several microns. Despite their large dimensions, however, such RNP granules are not surrounded by a delimiting membrane, an observation that has puzzled researchers for some time.

To investigate mechanisms of RNA localization in the multinucleate filamentous yeast *Ashbya gossypii*, a close relative of *Saccharomyces cerevisiae*, Lee et al. (2013) employ diverse single-molecule imaging techniques and sophisticated image analysis tools. By single-RNA in situ hybridization, they observed that the G1 cyclin-encoding *CLN3* transcript, unlike several other cell-cycle-related transcripts, showed subcellular clustering. Building on previous knowledge, the authors hypothesized that the heterogeneous *CLN3* localization may be regulated by the RNA-binding protein Whi3. Indeed, *CLN3* mRNA was randomly distributed in cells lacking a functional copy of Whi3. The distribution was also randomized in cells that expressed Whi3 variants deficient for an RNA-binding RRM motif or a polyglutamine stretch. To further investigate the role of Whi3 in mRNA localization, the authors measured the diffusion behavior of Whi3 using fluorescence correlation spectroscopy. Whi3 showed a considerably slower diffusion (>100-fold slower) than expected by its molecular weight. In agreement with this, Whi3 displayed a heterogeneous localization pattern, suggesting that Whi3 and *CLN3* assemble into large diffusion-limited complexes. The authors further demonstrate that the clustered *CLN3* distribution is required for the independent cycling of individual nuclei in a shared cytoplasm in the yeast. This is a remarkable finding with implications beyond multinucleate cells. Subcellular variability is a desired property of many biological processes, suggesting that similar clustering mechanisms could be used in other biological settings.

This study makes an important contribution to the field of RNA localization, but there is one elephant in the room: the molecular mechanism by which Whi3 spatially constrains *CLN3*. Lee et al. (2013) propose that *CLN3* clustering is mediated by conformational conversions of a misfolding-prone glutamine-rich domain. Glutamine- and asparagine-rich (Q/N-rich) sequences can self-assemble by prion-like or amyloid-like protein misfolding (Alberti et al., 2009; Han et al., 2012; Kato et al., 2012). Initially without structure, such sequences undergo conformational conversions into ordered states with remarkable self-templating abilities. These templates can induce the conformational conversion of other domains of similar sequence, thus setting in motion a chain reaction that culminates in macromolecular assembly. Although such a mechanism is appealing, it is not known whether it is the normal mode of action of Q/N-rich domains in living cells. Moreover, amyloid-like regulation of protein activities is associated with problems such as limited controllability and the danger of escalating into disease states.

Can we envision other ways of how Q/N-rich domains could promote macromolecular assembly? Recent findings suggest that RNA granules have liquid-like properties and form by liquid-liquid demixing phase separation (Brangwynne et al., 2009). Such phase-separated states seem to be based on multivalent interactions between multidomain RNA-binding proteins and RNAs (Li et al., 2012). Although the universality of this model has not been tested, it is very attractive because it is consistent with the dynamic, self-organized nature of RNP granules. Thus, in an alternative scenario, Q/N-rich domains would undergo ordinary protein-protein or protein-RNA interactions to promote RNP granule assembly (Malinovska et al., 2013; Schaefer et al., 2012) (Figure 1). Consistent with this scenario, many Q/N-rich sequences overlap with predicted protein-protein interaction motifs such as those for the formation of coiled coils (Fiumara et al., 2010). A quick analysis using the COILS server (http://embnet.vital-it.ch/software/COILS_form.html) indeed reveals a coiled-coil domain in Whi3 that precisely overlaps with the Q-rich sequence stretch. This suggests that other, more mundane mechanisms could also account for the observed clustering of *CLN3*.

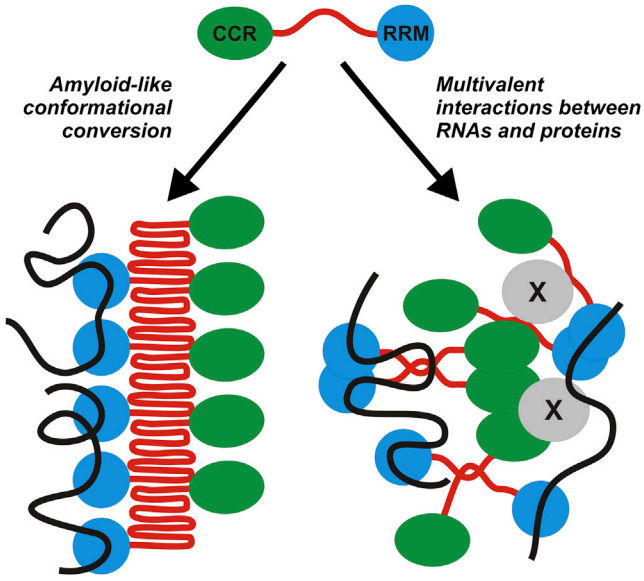

Figure 1. Two Possible Models for the Mechanism of *CLN3* mRNA Clustering by Whi3

Left: clustering of two *CLN3* mRNAs (black) is driven by self-assembly of the glutamine-rich domain (red). Right: spatial sequestration of the *CLN3* mRNAs is induced by multivalent interactions with Whi3 and other crosslinking factors (X). The latter model could rely on diverse protein-protein and protein-RNA interactions, such as Whi3 oligomerization via coiled coils or interactions between the polyglutamine stretch and RNAs or proteins.

Differentiation between these two models would require rigorous biochemical and biophysical studies to analyze the conformational states of Whi3 in vivo and in vitro, admittedly not an easy task. However, the data of Lee et al. (2013) already contain some hints that argue against an amyloid-like aggregation model. For example, fusion of the Q-rich stretch to GFP only mildly impaired GFP diffusion behavior, rather than the much stronger effect expected if the underlying mechanism were amyloid-like. This suggests that the Q-rich stretch only functions in its natural context, potentially because it acts synergistically with other domains (Figure 1). Consistent with this, *CLN3* clustering was absent in strains expressing RRM-deficient Whi3 but only slightly reduced in a Whi3 variant lacking the Q-rich domain.

To provide further support for an aggregation mechanism, the authors point to the similar subcellular distribution pattern of Whi3 and its isolated Q-rich domain. However, the apparent similarity of two localization patterns is not a strong argument for a common mechanism. Because an amyloid-like mode of assembly would predict incorporation of the Q-rich domain into native Whi3 complexes, a colocalization experiment with Whi3 and its isolated polyglutamine domain may be more informative. Additional experiments to determine the subcellular distribution and diffusion behavior of RRM-deleted Whi3 would also provide valuable mechanistic insight.

Why does the underlying molecular mechanism matter? It matters because the two models make fundamentally different predictions regarding the biology of the system. Amyloid-like aggregation of Whi3 would involve the formation of a potent template, which—if left uncontrolled—would grow into a single cytoplasmic diffusion sink for *CLN3*. Thus, in an amyloid-like aggregation model, sophisticated control mechanisms would have to be in place to generate the observed heterogeneous *CLN3* distribution. The model of locally condensing liquid-like states, on the other hand, seems more parsimonious, because such mechanisms do not need to be invoked to account for mRNA clustering. The underlying mechanism is also important because RNA-binding proteins are frequently associated with Q/N-rich sequences, suggesting that these domains may have general roles in RNP granule assembly. Ultimately, however, it matters because scientific progress is driven by competing models, and having only one model in mind may blind us to the underlying reality.

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